presumably to a wide variety of couplings to the membrane. We provide viscosity measurements for lipid bilayers with various compositions in the gel and liquid crystalline phases.

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Mc Model of Lipid Raft Protein Diffusion Matched to Live Cell Measurements with Controlled Chemical Perturbation Experiments

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Partitioning of certain type extra-cellular or cytosolic leaflet membrane proteins into functioning cholesterol stabilized domains within both leaflets of the membrane structure has long been shown by various studies facilitating different techniques. We have modeled how lipid-stabilized nano-domains influence the diffusion of some membrane proteins. By combining these simulations with non-perturbative experimental measurements of the diffusion behavior for chemically unperturbed and controlled perturbation cases of that diffusion, we were able to quantify the essential parameters describing the domains. The simulations feature four independent parameters, i.e. area fraction of the membrane occupied by domains, size of the domains, probability of the proteins to exit the domains, and effective protein diffusion within the domains. Kinetic Monte-Carlo modeling of proteins' association with membrane nano-domains has been performed over a selected region of the parameter space. Some of these parameters may be modulated at the single cell level during the time of a single experiment, allowing precise matching of all four parameters. We have used our non-perturbative imaging based FCS, bimFCS, to measure the diffusion of several membrane proteins from inner and outer leaflets of the cell-membrane over multiple length scales simultaneously. This allows extracting information of the domains which transiently trap the diffusing proteins.
We studied GFP-tagged GPI anchored proteins for the external leaflet and compared measurements of monomers and induced dimers on the same cells. For the intracellular leaflet, we studied Lyn-anchored GFP and used the Rapamycin induced cross-linking of the FKBP12 and Frb domains. We were able to successfully match experimental data with in-silico modeling by Kinetic Monte Carlo simulations, giving insight about sizes and area fraction of raft domains for each leaflet of the membrane, and also affinity of associated proteins with them.

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High-Speed Interferometric Scattering Microscopy of Receptor Mobility Reveals Anomalous Diffusion in Model Membranes

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The diffusive behavior of membrane proteins has been reported to change with the timescale of the measurement. High-speed single-particle tracking studies have revealed that it is Brownian on time scales less than 100 μs, confined from 1 to 10 ms, and Brownian at longer times (>10 ms).[1] This behavior has been interpreted as hop diffusion between submicron compartments in the plasma membrane. Due to the fundamental difficulty in achieving simultaneously high spatial and temporal resolution, these results have relied on a single experimental approach. Furthermore, attempts to use a complementary fluorescence technique to study anomalous diffusion have not reproduced the hop diffusion model.[2]
Here we use interferometric scattering microscopy (iSCAT)[3] to track the motion of the GM1 ganglioside receptor binding the B subunit of cholera toxin using 40-nm gold nanoparticle labels, in both supported lipid bilayers (SLBs) and droplet hydrogel bilayers (DHBs).[4] In SLBs, in single trajectories containing >200,000 frames and providing simultaneous 10 μs temporal and sub-nm spatial precision, we observe a shift from Brownian diffusion at early time scales (30 - 100 μs) to confined diffusion (100 μs - 10 ms) back to Brownian diffusion (>10 ms). In contrast, GM1 diffusion in DHBs is Brownian at all timescales. These data suggest that the presence of a structural support on one side of the bilayer is sufficient to produce anomalous diffusion, even for diffusers that do not penetrate the membrane.

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Investigation of Temperature Induced Mechanical Changes in Supported Bilayers by Reconstructed Atomic Force Microscopy Tapping Forces

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Membrane mechanics, such as rigidity, adhesion, and fluidity are known to play an important role in the regulation of many biological and cellular processes. These properties can be drastically affected by changes in temperature; increasing temperature can enhance membrane permeability and susceptibility whereas decreasing temperature is known to restrict membrane movement and the intake of essential nutrients. Temperature also plays a key role in determining the phase of a membrane; evidence of nanoscale rearrangement of lipids into raft-like domains has been found at physiological temperatures. Rafts are thought to be a site of importance for signal transduction and membrane protein trafficking. There is even a growing association between rafts and the development of neurodegenerative diseases. Thus, it is evident that temperature has a critical role in the maintenance of membrane biology. In this work, patches of total brain lipid extract bilayer containing 30% exogenous cholesterol were formed in phosphate buffered saline and examined using atomic force microscopy while the temperature was systematically increased from 28-40°C. Tapping mode atomic force microscopy (TMAFM) and scanning probe acceleration microscopy (SPAM) were used to obtain topographical and mechanical information of the sample, respectively. SPAM is relatively new technique that offers the advantage of quickly obtaining mechanical information, while maintaining nanoscale spatial imaging resolution, due to its basis in TMAFM. The principle of SPAM is that the noisy deflection signal is captured during TMAFM imaging and reconstructed to reveal the time-resolved forces between the cantilever tip and the sample surface. These tip/sample forces can be correlated with surface properties such as rigidity and adhesion. With SPAM it is also possible to construct harmonic images, thus providing further insights on subtle surface characteristics.

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Red Blood Cell Membrane Fluctuations and their Mechanisms: Passive Versus Active

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Red blood cells (RBCs) are soft and flexible biconcave discs, which are able to pass through capillaries with diameters several times smaller than the RBC size. The RBC deformability also results in noticeable membrane fluctuations, which must be correlated with RBC membrane properties. However, it is still under debate whether RBC membrane fluctuations are simply passive thermal undulations or whether a red cell also experiences active fluctuations which are driven by a metabolic activity or other cell processes. We will present direct evidence that the RBC undulations are not solely passive thermal fluctuations, which has been obtained from a set of different experiments and simulations using a high spatiotemporal resolution: from 10 microseconds to several seconds in time and up to 20 nanometers in space. Experimental results show a violation of the fluctuation-dissipation theorem (FDT) for freshly prepared RBCs indicating the existence of active processes. However, the FDT is satisfied for starved cells demonstrating that the membrane fluctuations are passive when the energy supply is absent. Experiments also show a considerable change in the fluctuation amplitudes for fresh and starved cells. Subsequently, we perform simulations which fully mimic and quantify the experiments. We are able to quantitatively extract RBC membrane properties including shear elasticity, bending rigidity, and membrane viscosity. Furthermore, we test several models for active fluctuations, which mimic different possible mechanisms including spectrin network remodeling, ion pumps, and change in the spontaneous membrane curvature. Simulation results agree well with experimental data and suggest that several processes mentioned above may contribute to active RBC fluctuations. We will discuss which processes are more likely to take place.

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Combined Stopped-Flow and Electrophysiological Experiments Suggest Direct Sodium Channel Inhibition by Model Fluorobenzene Anesthetics

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General anesthetics are often proposed to affect membrane properties through interactions with the lipid bilayer. Though recent studies have shown that inhaled anesthetics can interact more specifically with certain membrane proteins such as ion channels. For example, volatile anesthetics inhibit voltage-gated sodium channels to reduce neurotransmitter release. Fluorobenzenes (FBs), once considered for clinical use, were abandoned due to their flammability and toxicity but are still valuable model anesthetics for investigating the molecular mechanisms of anesthetics. We examined the properties of four FB compounds, 1,2-DiFB, 1,4-DiFB, 1,3,5-TriFB and HexaFB on lipid bilayer

and sodium channel function at equipotent clinically relevant concentrations. Effects on lipid bilayer properties were tested using a gramicidin channel based stopped-flow fluorescence assay for lipid bilayer perturbation; effects on sodium channel function were tested using whole-cell voltage-clamp electrophysiology on neuronal cells (ND7/23). The stopped-flow results showed that all four FBs minimally affected lipid bilayer properties, whereas the sodium channels were strongly inhibited by all four anesthetics. Inhibition of peak sodium current was voltage-dependent as a pre-pulse to a voltage at which half the channels were in the fast inactivated state ($V_{1/2}$) revealed strong inhibition compared to a pre-pulse to a voltage at which the majority of the channels were in the resting state (V_0). The FBs produce a left-shift in the voltage of half-maximal inactivation ($V_{1/2}$, also known as h_∞ or availability), with 1,2-DiFB showing the greatest and HexaFB the least shift; these changes are comparable to those observed with modern inhaled anesthetics such as isoflurane. Together these results suggest that these compounds alter sodium channel function through direct interactions with the channels, though we cannot exclude that membrane effects may become involved at high, supra-pharmacological concentrations.

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Relationship between Membrane Fluidity Changes, Phospholipid Protrusion Probability and Phospholipase A_2 Activity during Thapsigargin-Induced Apoptosis

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Secretory phospholipase A_2 exhibits much greater activity toward apoptotic versus healthy cells. Various plasma membrane changes responsible for this phenomenon have been proposed, including biophysical alterations described as ''membrane fluidity'' and ''order.'' Understanding of these membrane perturbations was refined by applying studies with model membranes to fluorescence measurements during thapsigargin-induced apoptosis of S49 lymphoma cells using probes specific for the plasma membrane: Patman and trimethylammonium-diphenylhexatriene. Alterations in emission spectra or anisotropy of these probes corresponded with enhanced susceptibility of the cells to hydrolysis by secretory phospholipase A_2. Furthermore, these alterations appeared to correlate temporally with fragmentation of actin filaments detected by confocal microscopy of phalloidin fluorescence. By applying a quantitative model, additional information was extracted from the kinetics of Patman equilibration with the membrane. Taken together, these data suggested that the phospholipids of apoptotic membranes display greater spacing between adjacent headgroups, reduced interactions between neighboring lipid tails, and increased penetration of water among the heads. The phase transition of artificial bilayers was used to calibrate quantitatively the relationship between probe fluorescence and the energy of interlipid interactions. This analysis was applied to results from apoptotic cells to estimate the frequency with which phospholipids protrude sufficiently at the membrane surface to enter the enzyme's active site. The data suggested that this frequency increases 50-100-fold as membranes become susceptible to hydrolysis during apoptosis.

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Differential Oligomeric Nature of mEos2 and mEos3.2 Fluorescent Proteins is Consequential to Diffusion and Confinement of Membrane Probes

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Lipid-mediated membrane heterogeneity is proposed to be an important organizing principle in mammalian cells. using super-resolution fluorescence localization imaging we track the diffusion of a large panel of fluorescent membrane probes ranging in size, mode of membrane anchoring, and putative phase-association. Our expressed probes include fluorescently-tagged palmitoylated or non-palmitoylated versions of transmembrane domains including linker of activated T-cell and haemagglutinin, and fluorescent protein anchored by GPI, palmitate-myristoyl moieties, or a geranyl-geranyl moiety. The recent advent of photoconvertible fluorescent protein mEos3.2, a ''truly monomeric'' mutant of its mEos2 predecessor1, has enabled us to compare directly monomeric and oligomeric versions of the same probes. Our results indicate that in some cases rates of diffusion are more than two fold lower in the mEos2 probes when compared to their mEos3.2 counterparts. Many mEos2-labeled probes also differ from mEos3.2-labeled probes in their exponents of anomalous diffusion. We attribute changes in mobility to the proteins' differential propensity to oligomerize, and are using the clustering effect of mEos2 to investigate whether phase heterogeneity influences cluster mobility.

1 Zhang, M. et al. Rational design of true monomeric and bright photoactivatable fluorescent proteins. Nat Methods 9, 727-729. (2012).

2198-Pos Board B217

Super-Resolution Imaging of T Cell Triggering Supports the Kinetic Segregation Model in the Adaptive Immune Response

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Among the most contentious questions in immunology is: how is the T cell receptor (TCR) triggered? using a combination of different single-molecule tracking, novel calcium signalling assays, crystallography and super-resolution imaging techniques we have demonstrated evidence to support the kinetic segregation model as the molecular mechanism behind the adaptive immune system.

The phosphorylation of the T cell receptor is required for T cell activation. The kinetic segregation model predicts that this is achieved by the spatial partitioning of important components such as the TCR and Lck kinase from the CD45 phosphatase according to the difference in size of the extracellular domain of the proteins on the cell membrane. We test this prediction by imaging the formation of contacts between T cells with a model antigen presenting cells in early stage contacts at endogenous expression levels. The approach allows the interrogation of the kinetic segregation with other models based upon aggregation or conformation change of the TCR. We present the results of this study.

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Properties of Membranes Derived from the Total Lipids Extracted from the Human Lens Cortex and Nucleus

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The organization and physical properties of lens lipid membranes made from the total lipids extracted from the human lens cortex and nucleus of 41- to 60-year-old donors were investigated using electron paramagnetic resonance (EPR) spin-labeling. Profiles of the phospholipid alkyl-chain order, fluidity, oxygen transport parameter, and hydrophobicity were assessed across membranes and coexisting membrane domains. Lens lipid membranes prepared from the lens cortex and nucleus were found to contain two distinct lipid environments, which were assigned as the bulk phospholipid-cholesterol domain and the cholesterol bilayer domain (CBD). The alkyl chains of phospholipids were strongly ordered at all depths, indicating that the amplitude of the wobbling motion of alkyl chains was small. However, profiles of the membrane fluidity, which explicitly contain time (expressed as the spin-lattice relaxation rate) and depend on the rotational motion of spin labels, show relatively high fluidity of alkyl chains close to the membrane center. Profiles of the oxygen transport parameter and hydrophobicity have a rectangular shape with an abrupt change between the C9 and C10 positions, which is approximately where the steroid ring structure of cholesterol reaches into the membrane. The amount of CBD was greater in nuclear membranes than in cortical membranes. The presence of the CBD in lens lipid membranes, which at 37°C showed a permeability coefficient for oxygen about 60% smaller than across a water layer of the same thickness, would be expected to raise the barrier for oxygen transport across the fiber cell membrane.

Protein-Lipid Interactions II

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Structural and Dynamical Changes for different types of Lipid Bilayer by Different Length of Poly-L-Lysine: MD Simulations

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Poly-L-lysine (PLL), a cationic polypeptide, is toxic to a variety of cell types (Nano letters 8, 2008; Nano letters 5, 2005) and is also an ideal vector for gene delivery (Pharmaceutical Research 17, 2000;Molecular Therapy 6, 2002) by forming pore in cell membrane and letting small molecules passing through membrane with it. Molecular mechanisms for the controversial role of PLL in cell membrane remain unclear. We investigated the molecular interactions of poly(L-lysine) at two different lengths with three different types of lipid bilayer: 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylserine (POPS) and